Insect herbivory on two willow species in northern Europe is independent of pollution load

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Pollution has repeatedly been suggested to increase plant susceptibility to insect attack, some diseases and severe climatic conditions. This study explored the effects of industrial pollution on leaf damage by insect herbivores (primarily defoliators) in two willow species, Salix myrsinifolia and S. caprea, growing naturally at different distances from two nickel-copper smelters (Harjavalta, SW Finland and Monchegorsk, NW Russia). The relationships between pollution load and herbivory were variable, from significantly positive to significantly negative. However, this variation was not related to the differences between polluters, willow species or study years; an overall effect of pollution on herbivory, as evaluated by meta-analysis of 12 individual data sets, did not differ from zero. This result hints that an overall increase in insect herbivory with industrial pollution, discovered in meta-analysis of published observational studies, may be an artefact caused by research and publication biases.

Introduction

Pollution has repeatedly been suggested to increase plant susceptibility to insect attack, some diseases and severe climatic conditions (Smith 1974, Baltensweiler 1985, Führer 1985, Hain 1987, Koricheva and Haukioja 1995). This suggestion is partially based on the hypothesis that abiotic stress increases plant nutritional quality for herbivores (White 1974, 1984). Insect herbivory can decrease plant growth, reproduction and survivorship (Hendrix 1988, Hawkes and Sullivan 2001, Zvereva et al. 2010, 2012), so further enhancement of the adverse consequences of pollution on plant fitness would be expected in a positive feedback fashion by an increased herbivory (Loehle 1988).

Importantly, most evidence supporting positive effects of industrial pollution on invertebrate herbivory is based on changes in herbivore densities along pollution gradients. Nonetheless, direct measurements of foliar losses to herbivores are surprisingly scarce. In particular, the published studies have yielded only 17 effect sizes for plant damage caused by insect herbivores, compared with 142 effect sizes for abundances of individual herbivore species (Zvereva and Kozlov 2010). Although herbivore densities are roughly proportional to the amount of damage they impose on plants, the damage can only be predicted if density is measured for the entire community of insect herbivores, which is rarely the case. This gives special importance to studies that explore changes in the proportion of leaf area consumed...
by herbivores along pollution gradients (e.g., Belskaya and Vorobeichik 2013).

The present study was designed to test the hypothesis that foliar damage caused by insect herbivores in two species of willows (Salix myrsinifolia and S. caprea) is positively associated with the level of industrial pollution. This hypothesis may seem currently very simplistic and outdated, but accumulation of data on plant losses to insects in pollution gradients is still needed to examine whether the meta-analysis of earlier studies, which demonstrated that herbivorous insects generally benefit from pollution (Zvereva and Kozlov 2010), may have overestimated this effect due to research and publication biases.

### Material and methods

#### Study areas

The copper–nickel smelters at Harjavalta (HA) in southwestern Finland (61°19’N, 22°09’E) and at Monchegorsk (MO) on the Kola Peninsula, NW Russia (67°56’N, 32°49’E) are situated in the boreal forest zone and emit predominantly SO₂ and heavy metals. However, the smelter in HA has affected natural ecosystems to a lesser extent than the smelter in MO, as HA is still surrounded by forests, whereas MO, even in the 1980s, was surrounded by extensive industrial barrens and dead coniferous forests.

The annual emissions of sulphur dioxide from HA decreased from ca 3.0 × 10⁷ kg in the mid-1940s to 4–5 × 10⁶ kg by the early 1990s; emissions of metals declined from 3.05 × 10⁵ kg of copper and 7.3 × 10⁴ kg of nickel in 1986 to 5.0 × 10⁴ kg of copper and 7 × 10³ kg of nickel in 1993 (Kozlov et al. 2009). Owing to relatively low factory chimneys, the deposition gradient is rather steep but relatively short. In the early 1970s, the zone of pine forest death covered ca. 1 km², and the zone of visible forest damage 5 to 6 km² (Laaksovirta and Sivola 1975). Since the mid-1990s, the emissions of HA have not acidified the soils in surrounding forests any more (Derome and Lindroos 1998), and thus the recently occurring detrimental effects are mostly caused by the pool of heavy metals accumulated in soils during the past decades.

The annual emissions of SO₂ from MO had reached a maximum of 2.78 × 10⁸ kg in 1983, steadily declined to 9.8 × 10⁷ kg in 1994, dropped to 4.5 × 10⁷ kg in 1999 and have remained at about this level since then. Metal emissions during the 1980s–1990s amounted to 3–8 × 10⁶ kg of nickel and 2–6 × 10⁵ kg of copper annually. Forests at a distance of up to 6 km from the smelter had perished already by 1946. From 1955 to 1963, the forest death area spread beyond 6–7 km from the smelter (Kozlov and Barcan 2000). Observations in the early 1990s revealed forest death in an area of 400 to 500 km², and the visible injuries to conifers were detected up to 50–60 km away from the smelter; the total area affected by emissions was estimated to exceed 10 000 km² (Kozlov et al. 2009).

#### Study plants

Willows were selected for this study because (i) they tolerate high pollution loads and therefore tend to become dominant in heavily polluted habitats, including industrial barrens (Kozlov and Zvereva 2007); (ii) they are used in reforestation of industrially polluted habitats (Kozlov et al. 1999, Isaeva and Belova 2012) and in phytoremediation of polluted soils (Jensen et al. 2009), thus the knowledge on pollution effects on herbivory is needed for planning rehabilitation measures; (iii) fumigation with SO₂ (at concentrations similar to those occurring near the smelter in MO) improved the quality of S. myrsinifolia foliage for the leaf beetle Chrysomela lapponica (Kozlov et al. 1996), indicating that industrial pollution may favour willow-feeding herbivores.

Salix caprea is a common willow species in both study areas; S. myrsinifolia ssp. borealis (referred to as S. borealis in several earlier studies) is abundant around MO but has not been found at our study sites around HA. In our study region, these willows grow as low to moderately high (4–7 m tall) trees (S. caprea) or high (2–3 m, exceptionally up to 5 m tall), sometimes tree-like, bushes with a few separate stems (S. myrsinifolia). Physiological and morphological responses of both willow species to pollution and herbivory were documented in earlier publi-

Study sites

The measurements were conducted at 10 sites around each smelter (Fig. 1). The concentrations of pollutants decrease hyperbolically with distance from the polluter, so the among-site distances were shorter close to the smelters. Co-ordinates of study sites are listed in Kozlov et al. (2009); environmental characteristics of the study sites were summarised by Zvereva and Kozlov (2001).

Around HA, the sites were chosen 0.5 to 15 km N-NW and 0.5 to 13 km SE-S of the smelter, along the road from Kokemäki to Nak-kila, except for the most distant site located between Järvikylä and Haistila. Plots were set up in secondary plant communities evolved on places that had earlier (presumably) been occupied by dry pine forests. Twenty-five juvenile genets of *S. caprea* of about the same size (stem diameter, mean ± S.E., 17.9 ± 0.4 mm, height 204 ± 4 cm) were haphazardly selected at each site on 23 May 1997.

Around MO, the sites were chosen 1 to 15 km NE and 1 to 47 km S of the smelter, along the road from Murmansk to St. Petersburg. The larger extent of the study area to the south, as compared with that to the north-east, resulted from the spread of aerial pollution by predominantly northern winds during the summer. Plots were set up in localities representing different stages of pollution-induced deterioration of primary spruce-dominated forests. Twenty-five female genets of *S. myrsinifolia* of about the same size (stem diameter 16.9 ± 0.5 mm, height 105 ± 3 cm) were tagged at each site on 10–14 June 1997. At the same time, 15 female genets of *S. caprea* (stem diameter 24.1 ± 1.2 mm, height 166 ± 4 cm) were tagged in six of these ten sites.

Earlier, we demonstrated that the analyses based on correlations of the observed biotic effects with log-transformed distances from the polluter and with foliar concentrations of one of the pollutants yielded apparently the same conclusions (Kozlov et al. 2009). Thus, because the aim was to detect the effect rather than to explore the dose–effect relationships, the distance from the polluter was used in this study as a proxy of the pollution load. Foliar concentrations of metal pollutants at the study sites were published earlier (Kozlov et al. 2009).

Measurements of insect herbivory

Foliar damage was measured in five randomly selected genets of each species at each site, because other genets were subjected to defoliation treatments (Zvereva and Kozlov 2000, 2001). This sample size is typical for studies addressing background herbivory: 38% of 419 published studies on insect herbivory on woody plants were based on samples collected from 1–5 plants per study site, and 51% of these studies measured losses of leaf area from 1–25 leaves per plant.

The measurements were conducted by the ends of the growth seasons, but at least three weeks before the beginning of leaf fall (HA:...
28 August–5 September 1997 and 28 August 1998; MO: 2–3 August 1997 and 1–5 August 1998). The first index of foliar damage, the proportion of damaged leaves, was estimated in a sample of 100 leaves, haphazardly selected from the crown of each bush (the same observer evaluated all plants within the impact zone of each polluter). Later, for each willow individual, 25 vegetative shoots of average length were selected and the fourth leaf (counting from the base) on each shoot was classified as belonging to one of the following damage classes, according to the proportion of the leaf area that was consumed or damaged (galled, mined or skeletonised) by insects: intact leaves, 0.01%–1%, 1%–5%, 6%–25%, 26%–50%, 51%–75% and 76%–100%. The number of leaves in each damage class was multiplied by the median value of the damaged leaf area (i.e., 0.5% for the damage class 0.01%–1%), and the obtained values were averaged across a sample (including undamaged leaves) to obtain the second index of herbivory: the proportion of leaf area lost to (or damaged by) insects. Sap-feeding insects were infrequent (< 1 indiv./100 willow leaves), and their density was therefore not quantified.

**Data analysis**

The hypothesis of possible non-linearity in responses was rejected using the protocol described by Zvereva and Kozlov (2010); therefore, effects of pollution on herbivory were explored by calculating Spearman rank correlation coefficients between the site-specific values of both indices of foliar damage and the distances from the polluters.

The overall effect of pollution on herbivory was searched by meta-analysis of the data collected around the two polluters from the two willow species during the two study years. This method increases the statistical power of the overall conclusion over the statistical power of individual correlations between foliar damage and the distance from the polluter. The correlation coefficients were $z$-transformed and weighted by their sample sizes (i.e., by the numbers of study sites) using the standard procedure in the MetaWin program (Rosenberg et al. 2000) to calculate effect sizes (ES). The effect was considered statistically significant if its 95%CI did not include zero. Meta-analysis was performed using a random effects categorical model. The variation in the ES values within and among the classes of categorical variables was explored by calculating the heterogeneity indices ($Q_T$ and $Q_B$, respectively) and testing these against the $\chi^2$ distribution (Gurevitch and Hedges 2001).

**Results**

By the end of the growth season, a substantial proportion of willow leaves had been damaged by insects (Fig. 2). However, herbivory resulted in the removal of a relatively minor proportion of leaf area (Table 1). The majority of damage was imposed by defoliating insects who skeletonised the leaves or made holes in them. The damage by mining and galling insects was below 1% of the total foliar damage across all samples.

The proportion of damaged leaves and the proportion of consumed leaf area demonstrated variable relationships with the distances from the polluters, from significantly positive to significantly negative (Fig. 2). The overall effect of pollution on herbivory did not differ from zero (ES = –0.13; 95%CI = –0.64…0.33) and was homogeneous ($Q_T = 10.9, df = 11, p = 0.45$).

The two indices of leaf damage yielded apparently the same conclusions ($Q_B = 0.05, df = 1, p = 0.83$) and therefore further comparisons were restricted to the proportion of consumed leaf area. The relationships between the consumed leaf area and the distance from the polluter (used as a proxy of pollution load) did not differ between two study years ($Q_B = 1.39, df = 1, p = 0.38$), between the two willow species in MO ($Q_B = 3.14, df = 1, p = 0.32$) and between two localities for *S. caprea* ($Q_B = 0.11, df = 1, p = 0.99$).

**Discussion**

**Effects of pollution on losses of willow foliage to insects**

The ambient concentrations of SO$_2$, recorded at the most contaminated sites around MO,
improved the quality of *S. myrsinifolia* for the larvae of the leaf beetle, *C. lapponica*, but neither sulphur dioxide nor metal-contaminated willow leaves were toxic for this herbivore (Zvereva et al. 1995, Kozlov et al. 1996). Based on this information, higher foliar damage was expected in willows growing closer to the smelters, but the data did not support this expectation: only the loss of leaf area in *S. myrsinifolia* in 1998 followed the expected pattern (Fig. 2).

The pronounced variation in the relationships between insect herbivory on willows and the level of industrial pollution is one of the most interesting findings of the present study. This variation was not associated with differences between polluters, willow species or study years. There is indeed a possibility that this variation, to a certain extent, results from a relatively low accuracy of site-specific measurements, because they were each based on five willow genets only. However, earlier studies demonstrated that a large fraction of within-population variation in herbivory is explained by differences among plant individuals, and that individual trees within

**Table 1.** The average levels of foliar losses of two willow species to insect herbivores (mean ± SE) in impact zones of two copper–nickel smelters.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Species</th>
<th>Year</th>
<th>Number of sites</th>
<th>Damaged leaves (%)</th>
<th>Consumed leaf area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harjavalta</td>
<td><em>Salix caprea</em></td>
<td>1997</td>
<td>10</td>
<td>71.5 ± 3.4</td>
<td>5.54 ± 0.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1998</td>
<td>10</td>
<td>64.9 ± 6.7</td>
<td>6.67 ± 1.23</td>
</tr>
<tr>
<td>Monchegorsk</td>
<td><em>S. caprea</em></td>
<td>1997</td>
<td>6</td>
<td>11.3 ± 3.0</td>
<td>0.76 ± 0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1998</td>
<td>6</td>
<td>5.4 ± 1.5</td>
<td>1.97 ± 0.60</td>
</tr>
<tr>
<td></td>
<td><em>S. myrsinifolia</em></td>
<td>1997</td>
<td>10</td>
<td>30.4 ± 5.2</td>
<td>4.29 ± 0.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1998</td>
<td>10</td>
<td>30.2 ± 5.3</td>
<td>11.99 ± 3.18</td>
</tr>
</tbody>
</table>
a site were similarly ranked across study years in terms of their defensive chemistry (Riipi et al. 2004) and herbivore damage (Crawley and Akhteruzzaman 1988). Measurement of the same willow genets during two subsequent years served to minimise the impacts of this variation on the results of the present study. Therefore, the variation in the relationships between foliar losses and pollution load between the two study years, most likely, resulted primarily from a lack of synchrony in density fluctuations between several insect species feeding on the same host plant, as well as between spatially separated populations of the same species inhabiting different study sites around each of the polluters (Selikhovkin 1995, Zvereva et al. 2002, Zvereva and Kozlov 2006).

Importantly, the inability to reveal a consistent and significant effect of pollution on losses of willow foliage to insects did not arise just from the absence of effects, but occurred due to the diversity in patterns, yielding a zero overall effect. This finding demonstrates that one-year and single-species observations, which dominated the earlier studies (reviewed by Zvereva and Kozlov 2010), can easily generate misleading conclusions. For example, if I was to base the decision only on the leaf area consumed from *S. caprea* near MO in 1997, I would have to state that plant losses to insect herbivores decrease with pollution (Fig. 2). In contrast, based on the data on *S. myrsinifolia* collected near MO in 1998, I would have to conclude that these losses increase with pollution (Fig. 2).

**General pattern in herbivory along pollution gradients**

The absence of an overall effect of pollution on losses of willow foliage to insects is in no way surprising. For example, damage of mountain birch along the MO pollution gradient (Kozlov 1985) and of Scots pine across the border between Russia and Finland (Nevalainen et al. 1994) varied independently of pollution. Furthermore, data collected from impact zones of 16 industrial polluters demonstrated a slight but significant decrease in insect herbivory with an increase in pollution (Kozlov et al. 2009). A similar conclusion was recently reached by the study of herbivory on European aspen near the copper smelter in Revda (Belskaya and Vorobeichik 2013).

On the other hand, 68 of 84 publications reviewed by Riemer and Whittaker (1989) and 55 of 79 publications reviewed by Kozlov (1990) reported an increase in densities of herbivorous insects with pollution. This discrepancy between the patterns observed in published and original data hints at a selection against studies whose outcomes contradicted the hypothesis on an increase in herbivory with pollution (Smith 1974, Baltensweiler 1985, Führer 1985). This hypothesis dominated the scientific community at least until the early 1990s. A meta-analysis of data collected around industrial polluters demonstrated that a positive association between pollution and herbivory is mostly supported by earlier publications (1970s–1980s), whereas studies conducted in the 1990s–2000s yielded non-significant overall effects (Zvereva and Kozlov 2010). This temporal trend is consistent with the development of several other research paradigms: earlier studies tend to overestimate the effect by preferentially documenting the patterns that are consistent with the prevailing theory, whereas publication of studies reporting small effects or effects contradicting the currently favoured hypothesis is delayed (Jennions and Møller 2002, Leimu and Koricheva 2004). The publication of the present study, which had been delayed for over 15 years, represents a good example of the time-lag bias: in the late 1990s, the reviewers were quite sceptical of the conclusion regarding the absence of a pollution impact on plant losses to insects and they suspected methodological flaws rather than questioning the predictive power of the hypothesis that was dominating at that time.

To conclude, the finding of an absence of an overall effect of industrial pollution on losses of willow foliage to insects supports the hypothesis (Zvereva and Kozlov 2010) that an increase in insect herbivory with increasing industrial pollution, discovered by meta-analysis of published studies, may be an artefact caused by research and publication biases, such as preferential documentation of patterns that fit the expectations of the researcher and selection against studies.
reporting effects that contradict the currently favoured hypothesis.

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